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Attention: **EXAMINER: DAVID T. FOX**

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From: Thomas P. McBride
Patent Department - BB4F
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MESSAGE: **PLEASE DELIVER TO EXAMINER DAVID T. FOX**

Attached is an Amendment to the Patent Application Serial Number 931,492, filed November 17, 1986, entitled Chimeric Genes for Transforming Plant Cells Using Viral Promotors.

If all pages are not received, or if copies are illegible, please contact Judy Lorenz at 314/537-7291.

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PATENT
38-21(404)A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE THE APPLICATION OF:

ROBERT T. FRALEY AND STEPHEN G. ROGERS — GROUP ART UNIT: 184

SERIAL NUMBER: 931,492 — EXAMINER: DAVID T. FOX

FILED: NOVEMBER 17, 1986 — JUNE 8, 1990

TITLE: CHIMERIC GENES FOR TRANSFORMING PLANT CELLS USING VIRAL PROMOTERS

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington D.C., 20231 on June 8, 1990
Thomas P. McBride
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AMENDMENT

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

This is in response to the Official Action dated March 8, 1990. Claims 1-18 remain for consideration. Applicants' attorneys wish to thank the Examiner for the courtesy extended and the assistance provided during the Office Interview of this case on April 12, 1990.

Rejection under 35 U.S.C. §103

Claims 1-15 stand rejected under 35 U.S.C. §103 as being unpatentable over Anderson taken with Guilley et al. It is the position of the Patent Office that Anderson teaches plant cells transformed with DNA constructs comprising a viral promoter, a gene encoding the kanamycin resistance enzyme, portions of the

CaMV genome and T-DNA borders. Guilley et al. is asserted to illustrate the teaching of the identification of the 35S and 19S CaMV promoters and their relative strengths. The Patent Office asserts that it would have been obvious to one of ordinary skill in the art to utilize the method of T-DNA mediated plant transformation utilizing viral promoters as taught by Anderson and to incorporate the CaMV 35S or 19S promoters taught by Guilley et al. and that the promoter would continue to function in its known and expected manner. The position of the Patent Office is respectfully traversed and reconsideration requested.

The Anderson patent, whether taken singularly or in combination with the Guilley et al. reference, does not teach, suggest or disclose Applicants claimed invention nor does it provide the necessary motivation to lead one of ordinary skill in the art to produce Applicants' invention with a reasonable likelihood of success. Anderson discloses the alleged use of a mammalian viral promoter in a chimeric gene used to transform plant cells. The use of non-plant promoters as the regulatory region of a chimeric gene for expression in plants has been shown by other researchers to be incapable of driving expression of a gene in plant cells. This fact is made clear and supported in the Rogers' declaration filed with Applicants' December 19, 1989 response to the July 27, 1989 Office Letter and accompanying citations, especially the Caplan reference. The Caplan reference states on page 818, second column, first full paragraph:

> Tn7(40) and Tn5(11) were inserted in vivo into the T-DNA of Ti plasmids pTiT37 and pTiA6NC, respectively, and were found to be efficiently cotranscribed with the T-DNA. The genes encompassed by these transposons failed to be expressed, presumably because the eukaryotic transcriptional machinery of the plant did not recognize the promoter sequences of these prokaryotic genes. Further attempts to express heterologous eukaryotic genes, such as the yeast alcohol dehydrogenase gene (23) or genes from mammalian cells, such as ß-globin (44), interferon (45), and genes under control of the SV40 early promoter (46), showed that none of these genes was transcribed in plant cells. This suggests that specific transcription factors or signals that are required for their expression are

present only in the cells or specific tissues of their original hosts.

As Caplan et al. states, "genes under the control of the SV40 early promoter (46), showed that none of these genes was transcribed in plant cells." The SV40 early promoter is a viral promoter from the Papovavirus group of animal viruses. The citation accompanying this statement (46) mentions that the article providing data to support this research is "in preparation." A search for an article by the authors named in the citation turned up no such published article, but the conclusion is drawn that other researchers in the field were unable to utilize non-plant promoters to drive gene expression in plant cells. Thus, one of ordinary skill in the art was confronted with conflicting data as to whether or what types of viral promoters would or would not work in plant systems. Researchers experimenting with viral promoters therefore had no reasonable expectations of success as to what would or would not work in plant systems.

Additionally, reason to doubt the conclusions reached by Anderson as to the usefulness of an animal viral promoter in a plant cell existed. The Anderson patent offers as its proof that the Thymidine kinase (TK) viral promoter drove the expression of a gene encoding for kanamycin resistance by showing that "up to 5% of the treated protoplasts cleaved and produced cell masses with as many as 16 cells" in the presence of kanamycin. It is asserted that this does not show that the TK promoter did indeed promote the expression of the kanamycin resistance gene in plant cells because an assay at the 16 cell stage is premature and does not provide any meaningful data. It has been shown in Applicants' laboratories that a percentage of cells and indeed whole plants can grow in the presence of kanamycin even though the cells or plants had not been effectively transformed. Furthermore, no verifying data such as enzyme studies or Western blots or the like were provided by Anderson to verify his alleged results. Thus, considerable unpredictability existed in the art as to whether animal viral promoters would work if inserted into plants.

Other factors also support the proposition that the use of viral promoters in plants was unpredictable. The Rogers Declaration (previously submitted) discussed reasons why the function of an isolated heterologous promoter in a

chimeric gene was unpredictable at the time the invention was made. The Patent Office states that with respect to the statements of Dr. Rogers as to the unpredictability of viral promoter function, "the viruses cited by Dr. Rogers which require other trans-acting proteins encoded by other portions of the viral genome for efficient promoter function do not appear to be closely related to CaMV." While it is true that the viral promoters referred to in the Rogers declaration are promoters from bacterial viruses, these bacterial viruses were the only viruses that had been sufficiently studied as of 1983 upon which one could draw any sort of conclusions or from which to hypothesize as to how other viral promoters, such as plant viral promoters, would function. The parent of the instant application was filed on April 15, 1983 and it is the knowledge of those skilled in the art as of that date against which the issue of obviousness must be measured. Indeed, if these other viral promoters are so unrelated to CaMV promoters, how can the promoter used by Anderson, the thymidine kinase promoter from the Herpes Simplex Virus, be considered relevant to the use of a plant viral promoter as Applicants have shown? If the other viruses as described in the Rogers' declaration are irrelevant and provide no basis from which one skilled in the art could base any reasoning as to expectations of success, then neither can the teachings of Anderson since the TK promoter of Herpes Simplex Virus is wholly unrelated to CaMV promoters and to plant viral promoters in general.

The Patent Office's reliance upon the Guilley et al. reference does not provide the missing teachings or motivation necessary to render Applicants' claimed invention obvious. It is stated by the Examiner that "Guilley et al. demonstrates successful function of the CaMV 35S and 19S promoters when "small" fragments of the CaMV genome are used..." (emphasis added). The fragment used by Guilley et al. is a 2.5 kb fragment of the CaMV genome. (See Fig. 1 and discussion on page 679, right-hand column. The entire CaMV genome is about 8000 bp in length (see Guilley, p. 673, first paragraph). Thus, Guilley et al. used about 31% of the CaMV genome to test for *in vitro* transcription. This can not be characterized as a "small" CaMV fragment. Applicants' representative CaMV 35S fragment utilized a 190 bp fragment, as described on page 13 of the specification and as shown in Fig. 6, and the representative CaMV 19S fragment

utilized a 455 bp fragment as described on page 10 and as shown in Fig. 9 of the specification. Thus, there was no reasonable likelihood of success that Applicants' isolated 35S or 19S fragments of the CaMV genome would work in view of Guilley et al.

Guilley et al.'s use of the 35S and 19S promoter is also distinguishable from Applicants' use in that Guilley et al. describes *in vitro* transcription in HeLa cells. The HeLa cell transcription system is a mammalian cell lysate system which is not a plant cell environment. The fact that a large CaMV fragment can effect transcription in a HeLa cell lysate system provides no likelihood or expectation of success that a small CaMV fragment isolating the 35S or 19S promoter from CaMV would be effective as a promoter in a chimeric gene when incorporated into a plant cell's genome. In fact, the 2.5 kb fragment used in Guilley et al.'s Hela cell system is large enough for trans-acting factors, if there were any, to effect transcription. This would have lead one of ordinary skill in the art to believe that a trans-acting factor may have been necessary in CaMV, as has been shown in other viral promoters as discussed in the Rogers declaration. This also teaches away from a reasonable likelihood of success of the isolated 35S or 19S promoters of CaMV as described by Applicants.

Furthermore, Guilley et al. does not teach the "strength" of the CaMV promoters as asserted by the Patent Office. Guilley et al. teaches that a large fragment of the CaMV can be transcribed *in vitro* in a mammalian cell lysate system. This does not demonstrate or suggest that smaller, isolated fragments of the CaMV containing the 19S or 35S promoter region would function as a "strong" promoter in a chimeric gene incorporated into the genome of a plant cell. There is no quantitative data or suggestion that the 35S or 19S promoters of CaMV would be "strong" promoters when integrated into plant cells from the Guilley et al. article and such a conclusion is only reached through the inappropriate use of hindsight applying Applicants' showing of the strength of the promoter when incorporated into a plant's genome.

In view of the foregoing remarks it is clear that a <u>reasonable</u> expectation of success of isolated promoters from CaMV 35S or 19S is not found from the teachings of Anderson or Guilley et al. whether taken singularly or in

combination. Claims 1-15 are merely "obvious to try" because Anderson and Guilley et al. provide no indication as to what portion of the CaMV 35S or 19S region is critical or that its use in a chimeric gene incorporated into a plant genome would exhibit a "strong" promoter activity. Therefore, the claimed invention in Claims 1-15 are not rendered obvious and the §103 rejection should be withdrawn. Such action is respectfully requested.

Claims 16-18 have been rejected under §103 as being unpatentable over Anderson taken with Guilley et al. as applied previously and further in view of Zambryski et al. Zambryski et al. is asserted as teaching the regeneration of whole plants from cells transformed with a vector containing chimeric genes, T-DNA borders and deleted tumor genes. Nothing in Zambryski suggests a differentiated plant capable of expressing a polypeptide through performance of a sequence of cellular steps including transcription of a chimeric gene that includes a promoter selected from the CaMV35S or CaMV19S promoter. In light of the discussion above distinguishing Applicants' invention from the teachings of Anderson and Guilley, Zambryski does not add the sufficient teachings or suggestions to render applicants claimed invention obvious. Therefore it is believed that the §103 rejection as it pertains to claims 16-18 should be withdrawn. Such action is respectfully requested.

In view of the foregoing remarks, it is believed that this application is now in condition for formal allowance. Such action in the regular course of business is respectfully requested. If the Examiner believes a phone conference would be beneficial to the quick allowance of this case, he is requested to phone Applicants' attorney at the number listed below.

Respectfully submitted,

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